

PHENOTYPIC AND BEHAVIOURAL CHARACTERISTICS  
USED TO IDENTIFY WILD BUFFALO *BUBALUS BUBALIS*  
FROM FERAL BACKCROSSES IN NEPAL<sup>1</sup>

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(With two text-figures)

**Key words:** *Bubalus arnee*, *Bubalus bubalis*, feral buffalo,  
Kosi Tappu Wildlife Reserve, Nepal, wild buffalo

All remaining populations of the wild Asiatic buffalo are known to cross breed with domestic and feral forms living in the vicinity of the handful of reserves in which the species is found. Censuses of these animals done in various places in India and Nepal have been criticized because researchers have used various criteria to differentiate wild buffalo versus feral backcrosses, thus rendering population estimates unreliable. Due to the highly endangered status of the wild buffalo, there is an urgent need for a set of criteria that can be applied more broadly to distinguish wild from feral forms. This paper describes the phenotypic and behavioural characteristics used to census wild buffalo and feral backcrosses in Kosi Tappu Wildlife Reserve, Nepal, which contains the last Nepalese population of the species. It is hoped that other researchers in the region may find these field characteristics useful in identifying wild stocks in South and Southeast Asia, where they are still thought to occur. Ideally, in all cases, detailed genetic studies are needed to plan managerial interventions such as translocation projects. Given the expense of such studies, it is suggested that translocations can be planned using consistent field identification criteria, until such time as more detailed genetic work is done.

INTRODUCTION

The decline of all species of Asian wild cattle and buffalo resulted in the restructuring of the Asian Wild Cattle Specialist Group (AWCSG) in 1995, by the Species Survival Commission (SSC) of IUCN — The World Conservation Union. A background document (Read *et al.* 1995) and draft action plan (Hedges 1995) were prepared and a meeting at Khao Kheow Open Zoo in Chonburi, Thailand was held in July 1995 to conduct a Conservation Assessment and Management Planning Workshop for four species of cattle and four species of buffalo that occur in various Asian range states (Byers *et al.* 1995). From the formal talks and general discussion, it was apparent that there is little information on the former or current

status of the wild buffalo *Bubalus bubalis* [=*B. arnee*] (Groves 1981), and that all populations are endangered to critically endangered for many reasons (Srikosamatara and Suteethom 1994, Heinen and Srikosamatara 1996). Wild buffalo have recently been listed under CITES Appendix II as a result of the known threats to all wild populations (Anon. 1997).

Since the species has been in domestication for at least 4,500 years (Clutton-Brock 1989), even the historical geographic range is in question. It is known that wild buffalo occurred at least from peninsular India to Southeast Asia, but there is evidence of buffalo in the Indus Valley over 5,000 years ago (Nowak 1999) and the wild form may have occurred from Mesopotamia eastwards (Sinclair 1977). The populations in Sri Lanka (Eisenberg and Lockhart 1972), peninsular Southeast Asia, Borneo and Java (Hedges 1995) have come into question, as it is not known whether they are partly or wholly of

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feral origin. Wild Asiatic buffalo have probably been in decline for long periods of time (Daniel and Grubb 1966, Seshadri 1986). Current site locations of putative wild stock are western Thailand, East and Central India, southern Bhutan and southeastern Nepal (Corbet and Hill 1992) in several isolated reserves.

A major concern is that domestic and feral buffalo are completely inter-fertile with wild buffalo; thus the genetics of wild stock are in question and field censuses become difficult when feral and wild forms look similar. This is not an isolated case. Wild sheep and goats of several species (Shackleton 1997), Eurasian wildcats (*Felis sylvestris*; Nowell and Jackson 1996), yak (*Bos mutus*; Schaller 1998), African wild ass (*Equus asinus*; Duncan 1992), and wild pig (*Sus scrofa*; Oliver 1993) are all known to interbreed with domestic and/or feral forms. In all these cases, however, known wild populations exist that are not affected by domestic mixing. Buffalo, Bactrian camels *Camelus ferus* (Schaller 1998) and Ethiopian wolves *Canis simiense* (Sillero-Zubiri and Macdonald 1997), may be under greater threat, because domestic or feral forms (shepherd dogs *Canis familiaris* in the case of the Ethiopian wolf), have access to breeding with wild stock through most or all of the geographic range.

Besides genetic introgression, threats of disease transmission are high when domestic and wild forms intermingle; this has been raised as a conservation issue for a number of species. Since detailed genetic studies are expensive, there is need for consistent field identification criteria that can be generally applied to census wild stock.

The American Zoo and Aquarium Association recommended field censuses and clarification of the species/subspecies of Asiatic buffalo (Read 1999). Antibody research is also needed to test for common diseases for all populations. Among the putative wild populations, it was thought that the one in Kosi Tappu Wildlife Reserve, Nepal (the only extant

Nepalese population) has a good chance of containing some pure wild stock. This is also likely in some populations in Assam and Madhya Pradesh (Divakar 1977, Divakar and Bhushan 1988), although there is debate about the genetic integrity of the stocks (Choudhury 1994). In spite of its importance as the wild progenitor of the domestic buffalo, the species is poorly studied (Cockrill 1967). The introduced feral population in Australia is probably the best known (Tulloch 1970, 1978, 1979). Due to the endangered status and importance of this species worldwide, the Department of National Parks and Wildlife Conservation in Nepal (DNPWC) is considering translocation of buffalo from Kosi Tappu to one or more of Nepal's other lowland parks or reserves to secure the species. *Bubalus bubalis* is known to have occurred in Chitwan National Park until the 1960s (Seidensticker 1975) and probably once ranged throughout the Nepalese lowlands, including areas now protected (Fig. 1). The phenotypic and behavioural characteristics used for the census in Nepal are described herein. It is hoped that these criteria will also be useful to researchers studying the species elsewhere.

#### Description of the study area and previous research

Kosi Tappu Wildlife Reserve was established in 1976 and is spread over 175 sq. km. It is Nepal's only Ramsar site and contains extensive wetlands that are important stopover and wintering areas for waterfowl (Sah 1997). It is located on the floodplain of the Kosi River in Sunsari, Saptari, and Udayapur districts in southeastern Nepal (75 to 100 m above msl; Fig. 2). The reserve is subject to extreme flooding during monsoon; buffalo and other ungulates frequently leave at that time and seek refuge in croplands. About 80% of the land area is dominated by tall grasses such as species of *Saccharum*, *Phragmites* and *Typha*, and open river banks. Mixed forests of *Bombax*, *Dalbergia*



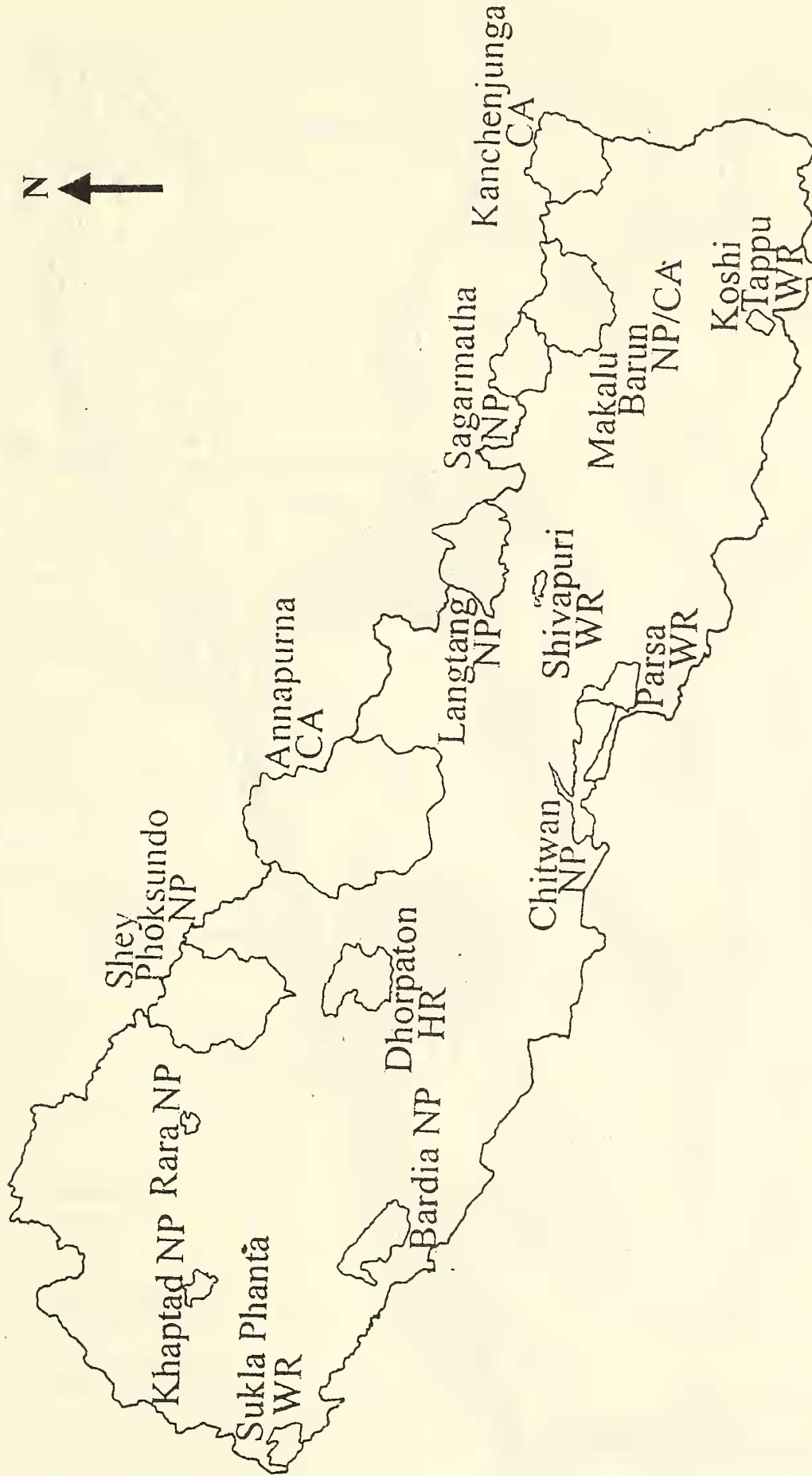


Fig. 1: Locations of the protected areas of Nepal  
(NP, National Park; WR, Wildlife Reserve; CA, Conservation Area; HR, Hunting Reserve.  
The five protected areas located along the southern border are all considered within the historical geographical range of wild buffalo.)

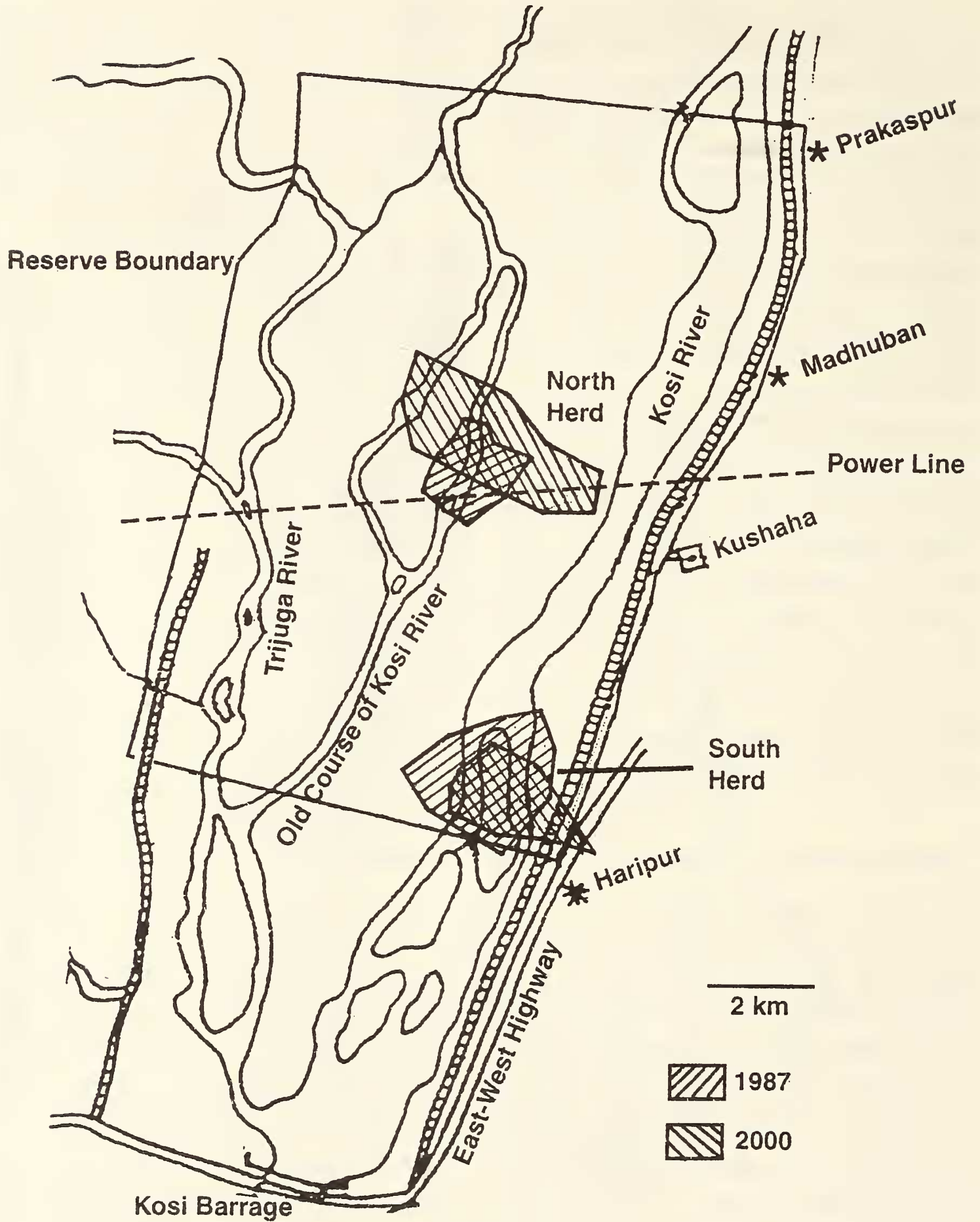


Fig. 2: Map of Kosi Tappu Wildlife Reserve, showing important locations around and within the boundaries

and *Acacia* make up the remainder. The vegetation was investigated by Dahmer (1978) and more extensively by Sah (1997); it is generally characteristic of riverine communities in the monsoonal climate of the Gangetic Basin (Stainton 1972).

The population of wild buffalo in Kosi Tappu has been censused numerous times and methods have been scrutinized because there are semi-feral as well as domestic buffalo that regularly backcrossed with wild bulls in the area (Mishra 1981, Shrestha 1981, 1997). Most of the censuses were conducted by local amateurs, or by biologists who spent only a week or two in Kosi Tappu, with little prior experience. There have been two longer studies: one in 1976 by Dahmer (1978) for one year, and the other by Heinen (1993a) from 1986 to 1988 covering 1.5 years. Heinen and Singh (*in press*) repeated the census in 2000, allowing for comparisons of population structure and growth rates over a 24-year period. Both the long-term studies considered flooding to be the major source of mortality and recommended translocations to Chitwan National Park. Heinen (1993a) considered the population not viable due to the probability of chance extinction (e.g. Goodman 1987). Census was completed from mid-February to early April, the season of grass cutting and burning, as the animals are more visible (Heinen 1993b) and also before the extreme heat of pre-monsoon set in, as the animals then become very inactive during the day.

The studies of Dahmer (1978), Heinen (1993a), and Heinen and Singh (*in press*) are considered to be the most definitive, as they were conducted by individuals who had spent extensive periods observing buffalo. However, given that backcrossing with wild males has been occurring in the region regularly since the malaria eradication in the 1950s (>40 years or >6 average buffalo generations, estimated at 6.5 years), one cannot be sure that animals counted as 'backcrossed' are not 'wild' in at least some

cases. It also may not matter, for animals backcrossed for so many generations. An f-6 backcross would be 98.44% 'wild' based on nuclear DNA  $100[1.0-0.5^6]$  (Falconer 1981). Such animals may be appropriate to include in a conservation breeding or translocation scheme, pending mitochondrial DNA analysis, provided that their appearance and behaviour are consistent with wild individuals. These issues are further addressed below.

### Phenotypic Characteristics of Wild Buffalo

Wild buffalo go through changes in pelage with age. Young (< 6 months) calves are buff in colour, and begin to darken in the first year. They retain lighter coloration into the second year, but the coat continues to darken until the females achieve adult coloration by the third year. Thus, young, juvenile and adult females can be distinguished on the basis of coloration, and overall body and horn size. Adult coloration is very dark, with noticeable whitish markings in several places on the body. Wild buffalo have one or two white chevron marks on the underside of the neck. They also have white hocks, white tail tips, and fine white markings around the eyes and along the sides of the nose and mouth. The white markings tend to become more apparent in adults, may fade with advanced age, and are not necessarily visible on new calves. In all cases, they are visible on late first year calves. Young males can be distinguished from adults into the third and fourth years; they are noticeably smaller and the pelage retains the light coloration. Adult males at prime breeding age (> 4 years) tend to be very black, except for the whitish markings described.

The horns of wild buffalo form wide, upwardly sweeping semicircles and lack appreciable curvature in the lateral vertical plane. Horns begin to grow early in life and are apparent from a distance in all calves by the age of 6 months. Horns on very young calves extend horizontally from the skull, and lack any curl.



As in most Bovids, horns grow throughout life and can be used as a proxy for age among adults. Since growth rates can vary based on nutrition, sex and dominance status of individuals, the censuses did not record age estimates for the adults. On an average, among adults, females tend to have longer horns than males, but males tend to have much thicker horns. Thus, if the genitalia are not visible (typically the situation in the tall grasses of Kosi Tappu and other places in which purely wild stock are thought to occur), adults can be sexed by viewing the horns alone. Bulls are noticeably larger and have massive musculature. First and second year calves are difficult to sex because it is usually not possible to approach wild herds close enough to see the genitalia. Thus, sex classes were assigned for adults (> 2 years old) only.

In contrast, the typical 'river' breeds of domestic buffalo common in South Asia are generally much darker throughout life and show few to none of the white markings described above (see Cockrill 1974). Hall and Ruane (1993) recognized 74 breeds or varieties of domestic buffalo (fewer than for any other major domesticated mammal); some breeds retain light-colored tail tips and hocks. They are smaller and their horns are usually very small with noticeable curvature along both horizontal and vertical planes. Thus, pure domestic buffalo can be told from wild buffalo rather easily in Nepal and Central India. Some lesser-developed breeds of domestic buffalo, especially the 'swamp' breeds more common in Southeast Asia (Cockrill 1974), retain a few wild phenotypic characters and many of the buffalo residing in Kosi Tappu and elsewhere are known to be backcrosses. Thus, there are many individuals that look essentially wild, although they are not pure. Other field criteria are, therefore, needed for identifying wild stock.

### Behavioural Characteristics of Wild Buffalo

Behavioural work is increasingly being

studied in conservation biology (Caro 1998) and several behavioural characteristics were used to differentiate between wild and backcrossed buffalo here. The literature suggests that wild buffalo females remain in the herds in which they were born, while males leave their natal herds, usually in or before their third year (Heinen 1993a). Herding behaviour can vary seasonally in Australia (Tulloch 1978), but this was not observed in Kosi Tappu females. Members of wild herds of females and their dependent offspring (called 'mixed' herds by Heinen 1993a, not to be confused with 'backcrossed' female herds) are thought to be highly philopatric. Again, this is not so in the feral Australian population (Tulloch 1970).

Dahmer (1978) described two mixed herds in Kosi Tappu in 1976, the north and south herds, and Heinen (1993a) relocated those herds in 1986/88 in highly overlapping home ranges with those mapped by Dahmer. Heinen and Singh (2000) relocated the herds again in 2000 and found that their home ranges had not changed appreciably from the previous work in spite of the fact that the main channel of the Kosi River had changed course (Fig. 2). Animals in mixed herds showed a great deal of phenotypic uniformity compared to those in backcrossed herds, and herd members were always seen in close proximity to each other in all three Kosi Tappu studies. Backcrossed herds were seen in variable groupings, sometimes with as few as 6 animals. Mixed herds were always seen with one herd bull in attendance; backcrossed herds were also generally seen with an attendant wild bull, but not necessarily so. Several matings, between wild bulls and feral backcrosses, were observed in 1986, 1988 and 2000 (Heinen, unpublished field notes).

Herding behaviour in single adult males in Kosi Tappu is highly variable. Younger males (up to four years, as estimated from horn length and body size) were frequently seen in small groups with other males (6-8 animals), whereas



older males were frequently seen alone or in pairs. Both Dahmer (1978) and Heinen (1993a) recorded larger herds of older males on occasion, and thus, this behavior may vary seasonally or even daily. Herd bulls are those that accompany female herds, and no more than one at a time has been observed attending any herd, be it mixed (wild) or backcrossed.

Another aspect of behaviour that can be used as a clue in identification is flight distance. The mixed herds counted as wild in Kosi Tappu were extremely wary of any human approach, be it on foot, boat, elephant, or in vehicles, and all counts had to be made from relatively long distances (>150 m and usually more) using high powered binoculars. Females in Kosi Tappu have been observed frequently using 'phalanx' behaviour to protect calves from approaching humans prior to fleeing, also described for wild type buffalo in Sri Lanka (Eisenberg and Lockhart 1972). Phalanx behaviour is defined as adult females forming a lateral line, each facing the intruder, with calves interspersed between the adults. The backcrossed herds were all relatively easy to approach in vehicles (<100 m) and most of them allowed walking humans to approach within c. 150 m or less. They tended not to form a phalanx before fleeing. These subtle behavioural differences imply that the semi-feral backcrossed herds are rounded up by their owners on occasion, probably annually, to collect first year calves (especially males) for market (Dahmer 1978 and Heinen 1993a). Local villagers told us that these animals could be lured with salt licks like feral cattle (*Bos indicus*) breeding in the reserve.

Herd bulls tended to flee with their females on human approach. In contrast, single adult wild bulls allowed fairly close approach, frequently within 100 m or less. In all such cases, they faced the intruder and stood their ground. If a human came too close, the single adult bulls were likely to take one or several steps forward. Closer approach could presumably lead to a charge:

several people have been injured or killed over the years in Kosi Tappu as a result of such encounters (Heinen 1993a). There are no records of wild or backcrossed females in Kosi Tappu charging humans directly.

#### DISCUSSION

Mixed herds of wild buffalo with all the aforementioned phenotypic traits, with little visible phenotypic variability among individuals, a consistent herd structure (when not disturbed), using consistent home ranges over long periods of time, and with behavioural patterns that showed phalanx formation and intolerance to human approach were censused in three different seasons in Kosi Tappu from 1976 to 2000. All other females and calves that looked wild were considered backcrossed, in spite of the fact that some individuals in backcrossed herds displayed all phenotypic traits of wild buffalo, and all individuals in those herds displayed most traits (Table 2).

These methods *may* tend to undercounting of the wild buffalo. For example, a record sized female buffalo in Kosi Tappu was alive during Heinen's 1986-1988 study. The animal displayed all the traits of a wild buffalo, but was consistently seen with a herd in which some members lacked

TABLE 2  
CENSUS RESULTS FOR BACKCROSSED  
BUFFALO HERDS CENSUSED DURING 2000

Herd	Adult Females	2nd year	1st year	Total
1.	25	9	6	40
2.	15	8	5	28
3.	8	1	3	12
4.	3	1	2	6
5.	4	2	1	7
6.	8	1	0	9
7.	5	2	2	9
8.	7	3	2	12
9.	5	2	1	8
Total	80	29	22	131

some wild phenotypic traits. Some females in this herd, for example, had distinctively more curl in the horns than was the case in the north and south herds, and some lacked a few of the characteristic white markings. Thus the female, which died of natural causes in 1994 and whose rack is now on display at the reserve headquarters, was not considered 'wild' (Heinen 1993a). Other methods, however, surely overcount wild buffalo. Suwal (1993) recorded a population of 158 in 1993, only 5 years after Heinen (1993a) recorded 93. This corresponds to a sustained population growth rate of >10% per year. Possible, but not likely for a large artiodactyl that generally produces a single offspring and usually calves biannually (Bronson 1989).

It is suspected that Suwal (1993) counted the large backcrossed herd seen regularly in the late 1980s (whose descendants were seen again in 2000; Herd 1 in Table 2) as 'wild' (the herd described above), and some members weren't based on phenotypic traits alone. Since females are thought to stay with their natal herds, it is likely that the matriarchal-line was of domestic origin and that the animals had been backcrossing with wild males for at least 6 generations. Since these animals acted essentially wild, and only a few minor phenotypic traits visible on some individuals were used to distinguish them from wild stock, it is possible that members of this herd could be used in a translocation program. However, that cannot be advised until mitochondrial DNA studies can be performed to determine how different the matriarchal-line really is from wild buffalo. Similarly, Chaudhary (1999) recorded a population of 174 wild buffalo in Kosi Tappu (in 1999), which is more likely than Suwal's (1993) estimate, but results from 2000 (Heinen and Singh, in press; Table 1) showed a decrease of 17% one year later in spite of many recent births. Evidence thus suggests that some studies were not long enough to allow researchers adequate

TABLE I  
POPULATION STRUCTURE OF WILD BUFFALO  
IN KOSI TAPPU RESERVE  
AND ANNUAL POPULATION GROWTH RATE

Year	Adult		2nd year	1st year	Total	Calves/ cows
	Male	Female				
1976	12	18	22	11	63	0.61
1987	32	29	14	16	91	0.55
1988	37	33	8	15	93	0.45
2000	56	53	17	19	145	0.36

$r_1$  (1976 to 1987) = 0.033,  $r_2$  (1987 to 1988) = 0.022,  
 $r_3$  (1988 to 2000) = 0.037,  $r$  total (1976 to 2000) = 0.035.  
1976 (Dahmer 1978), 1987, 1988 (Heinen 1993a) and  
2000 (Heinen and Singh, in Press)

time to familiarize themselves with all the characteristics described above to identify the wild stock.

Evidence still suggests (Heinen 1993a) that there is little chance of domestic genes entering the population of wild buffalo because there is little chance of a domestic male competing with wild males and monopolizing breeding in a mixed herd. Heinen and Singh (2000) saw three domestic males in the Reserve in March 2000, but all were grazing near villages along the boundary and far from any mixed or backcrossed herds. No wild male counted in these studies had any phenotypic or behavioural traits suggesting that it was backcrossed. Furthermore, local buffalo owners informed us that they do not keep domestic males with their herds because wild males will attack them, and that their goal was to crossbreed their females with wild males, as the backcrossed males command higher prices when sold as draught animals. There is no evidence, therefore, that either backcrossed domestic, semi-feral, or feral males eventually enter the breeding population in Kosi Tappu. Shrivastava (*pers. comm.*) received similar information from the local buffalo owners around Kaziranga National Park, Assam during a recent study (September, 2000 to January, 2001). Thus,



the evidence suggests that both the reserves contain populations of pure wild buffalo in addition to semi-feral and backcrossed herds from which male calves are removed.

#### CONCLUSIONS

Hedges (1995) discussed the need for genetic studies, both mitochondrial and nuclear, to assess differences among the putative wild, wild type, feral, semi-feral and domestic buffalo that occur throughout tropical Asia. The first major genetic study of wild buffalo is currently underway in Kaziranga National Park (Dr. P. Malik *pers. comm.*). Even if appropriate mitochondrial genetic markers that differentiate wild from backcrossed matriarchal lines are isolated, detailed study should also be conducted at other sites because wild buffalo in Assam are noticeably larger and considered to be a distinct subspecies from those in Central India or Nepal. The available evidence suggests that animals counted as 'wild' in Kosi Tappu were truly so, and some females and calves recorded as 'backcrosses' may also be wild, or at least backcrossed for enough generations to make the distinction arbitrary. Thus, the methods described here are conservative indicators of what constitutes a wild buffalo.

This species is threatened with extinction throughout its range. I suggest that translocations can be planned with the current information by focusing on animals from herds that display all phenotypic and behavioural characteristics of the wild form. There are numerous reserves throughout lowland Nepal and North and Central India that are within the known historical range

of the wild buffalo, but do not have populations. The Government of Nepal is considering a translocation proposal based on recommendations made from the identification criteria used here (Heinen and Singh, 2000). Furthermore, if future genetic work shows that some backcrossed feral females are essentially wild by nuclear DNA criteria, do not contain specific mitochondrial DNA markers that may render them less fit, and display most phenotypic and all behavioural traits consistent with wild buffalo, then such animals should also be considered for use in translocation projects.

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